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METHOD OF PRODUCING BIOLOGICALLY ACTIVE HUMAN ACIDIC FIBROBLAST...

Stegmann et al.

Appl. No.: Unknown Atty Docket: CVGENG.008CP1



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		BsaBI							
		BsaBI							
		BglIII	VspI	BfmI	MbiI	XbaI			
4951	CGGTAGAGGATCGAGATCTCGATCCCGCGAAATTAATACGACTCA	CTATAGGGGAATTGTGAGCGGATAACAATTCCTCTAGAAATAA							
	CGCATCTCCTAGCTCTAGAGCTAGGGCGCTTTAATTATGCTGAGT	GATATCCCCCTTAACACTCGCCTATTGTTAAGGGGAGATCTTTATT							
+2		MetAlaGluGlyGlu	IleThrThrPheThrAlaLeuThrGluLysPheAsnLeuProPr						
			HpaI						
			HindII					SmaI	
			HincII					AvaI	
5041	TTTTGTTTAACTTTAAGAAGGAGATATACATATGGCTGAAGGGGA	AATCACCACCTTTACAGCGTTAACGGAGAAATTTAACCTTCGCGC							
	AAAACAAATTGAAATCTTCTCTATATGTATACCGACTTCCCCT	TTAGTGGTGGAAATGTCGCAATTGCCTCTTTAAATTGGAAGGCGG							
+2	oGlyAsnTyrLysLysProLysLeuLeuTyrCysSerAsnGlyGly	HisPheLeuArgIleLeuProAspGlyThrValAspGlyThrAr							
	SmaI	PstI							
	AvaI	HindIII	BfmI	EcoRI				NruI	
5131	CGGGAATTACAAAAACCAAGCTTCTTTACTGCAGTAACGGAGG	ACACTTCCTGCGAATTCTGCCAGATGGCACAGTAGATGGGACTCG							
	GCCCTTAATGTTTTTTGGGTTCTGAAGAAATGACGTCATTGCCTCC	TGTGAAGGACGCTTAAGACGCTTACCGTGTCTATCCTGAGC							
+2	gAspArgSerAspGlnHisIleGlnLeuGlnLeuSerAlaGluSer	ValGlyGluValTyrIleLysSerThrGluThrGlyGlnTyrLe							
		Sali							
	PvuI	HindII						RsaI	
	NruI	PvuII	XmaIII	HincII				MlsI Csp6I	
5221	CGATCGCTCCGACCAGCACATTTCAGCTGCAACTCTCGGCCGAAAG	CGTTGGAGAGGTCTATATCAAGTCGACGGAGACTGGCCAGTACCT							
	GCTAGCGAGGCTGGTCTGTGTAAGTCGACGTTGAGAGCCGGCTTTC	GCAACCTCTCCAGATATAGTTCAGCTGCCTCTGACCGGTCTATGA							
+2	uAlaMetAspThrAspGlyLeuLeuTyrGlySerGlnThrProAsn	GluGluCysLeuPheLeuGluArgLeuGluGluAsnHisTyrAs							
	StyI								
	NcoI	Mva1269I	XbaI						
5311	TGCCATGGACACCGATGGGCTTCTGTATGGCTCAGACAGCCTAA	CGAAGAATGCTTGTCTTAGAAAGACTAGAAGAAAACCATTAACA							
	ACGGTACCTGTGGCTACCCGAAGACATACCGAGTGTCTGCGGATT	GCTTCTTACGAACAAAGATCTTTCTGATCTTCTTTTGGTAATGTT							
+2	nThrTyrIleSerLysLysHisAlaGluLysAsnTrpPheValGly	LeuLysLysAsnGlySerCysLysArgGlyProArgThrHisTy							
	RsaI								
	Csp6I	StuI							
5401	CACGTACATATCGAAAAACATGCAGAGAAGAACTGGTTGTGAGG	CCTTAAAAAAATGGTTCCTGTAAGCGTGGACCACGGACTCACTA							
	GTGCATGTATAGCTTTTTTGTACGTCTCTTCTTGACCAACATCC	GGAATTTTTTTTACCAAGGACATTCGCACCTGGTGCCTGAGTGAT							
+2	rGlyGlnLysAlaIleLeuPheLeuProLeuProValSerSerAsp	***							
		Sali	XmaIII						
	SacI	EcoRI SacI HindII	XhoI						
	MlsI	Ec1136II	BamHI	Ec1136I IHincII IHindIII	NotIAvaI				
5491	TGGCCAAAAGGCTATCTGTTCCTGCCACTACCGTAGCTCCGA	CTAAGGATCCGAATTCGAGCTCCGTCGACAAGCTTGC GGCCGCAC							
	ACCGGTTTTCCGATAGAACAAGGACGGTGATGGTCACTCGAGGCT	GATTCTAGGCTTAAGCTCGAGGACGCTGTTTCAACGCCGGCGTG							

CHEMICALLY SYNTHESIZED haFGF GENE AND
CORRESPONDING AMINO ACID SEQUENCE

FIG. 1

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FGF fr HUMECGFB	TTC	GCC	CTG	ACC	AAG	AAT	CTG	CCT	CCA
haFGF 155	TTT	GCG	TTA	ACG	AAA	AAC	CTT	CCG	CCC
No amino acid	009	011	012	013	015	017	018	019	020
	Phe	Ala	Leu	Thr	Lys	Asn	Leu	Pro	Pro
FGF fr HUMECGFB	AAG	AAG	AAA	CTC	CTC	TGT	AGC	GGG	GGC
haFGF 155	AAA	AAA	AAG	CTT	CTT	TGC	AGT	GGA	GGA
No amino acid	024	025	027	028	029	031	032	034	035
	Lys	Lys	Lys	Leu	Leu	Cys	Ser	Gly	Gly
FGF fr HUMECGFB	AGG	ATC	CTT	CCG	GTG	ACA	AGG	GAC	AGG
haFGF 155	CGA	ATT	CTG	CCA	GTA	ACT	CGC	GAT	CGC
No amino acid	039	040	041	042	046	049	050	051	052
	Arg	Ile	Leu	Pro	Val	Thr	Arg	Asp	Arg
FGF fr HUMECGFB	AGC	CAG	AGT	GCG	GTG	GGG	GTG	ATA	AGT
haFGF 155	TCC	CAA	TCG	GCC	GTT	GGA	GTC	ATC	TCG
No amino acid	053	060	062	063	066	067	069	071	073
	Ser	Gln	Ser	Ala	Val	Gly	Val	Ile	Ser
FGF fr HUMECGFB	ACC	TTG	GAC	TTA	TAC	ACA	CCA	AAT	GAG
haFGF 155	ACG	CTT	GAT	CTG	TAT	ACG	CCT	AAC	GAA
No amino acid	074	080	085	088	089	093	094	095	096
	Thr	Leu	Asp	Leu	Tyr	Thr	Pro	Asn	Glu
FGF fr HUMECGFB	TGT	TTC	CTG	AGG	CTG	GAG	GAG	ACC	TAT
haFGF 155	TGC	TTT	CTA	AGA	CTA	GAA	GAA	ACG	TAC
No amino acid	098	100	101	103	104	105	106	111	112
	Cys	Phe	Leu	Arg	Leu	Glu	Glu	Thr	Tyr
FGF fr HUMECGFB	TCC	AAG	AAG	AAT	GTT	CTC	AAG	AAG	GGG
haFGF 155	TCG	AAA	AAA	AAC	GTA	CTT	AAA	AAA	GGT
No amino acid	114	115	116	121	124	126	127	128	130
	Ser	Lys	Lys	Asn	Val	Leu	Lys	Lys	Gly
FGF fr HUMECGFB	AGC	TGC	AAA	CGC	GGT	CCT	CAG	AAA	GCA
haFGF 155	TCC	TGT	AAG	CGT	GGA	CCA	CAA	AAG	GCT
No amino acid	131	132	133	134	135	136	142	143	144
	Ser	Cys	Lys	Arg	Gly	Pro	Gln	Lys	Ala
FGF fr HUMECGFB	TTT	CTC	CCC	CTG	GTC	TCT	TCT	GAT	
haFGF 155	TTC	CTG	CCA	CTC	GTG	AGC	TCC	GAC	
No amino acid	147	148	149	150	152	153	154	155	
	Phe	Leu	Pro	Leu	Val	Ser	Ser	Asp	

MODIFICATION IN MOLECULE haFGF 155 CODONS.

FGF fr HUMECGFB—THE SEQUENCE FROM GENBANK(at NCBI), haFGF 155
—THE SEQUENCE SYNTHESIZED BY THE INVENTION AUTHORS.

FIG. 2

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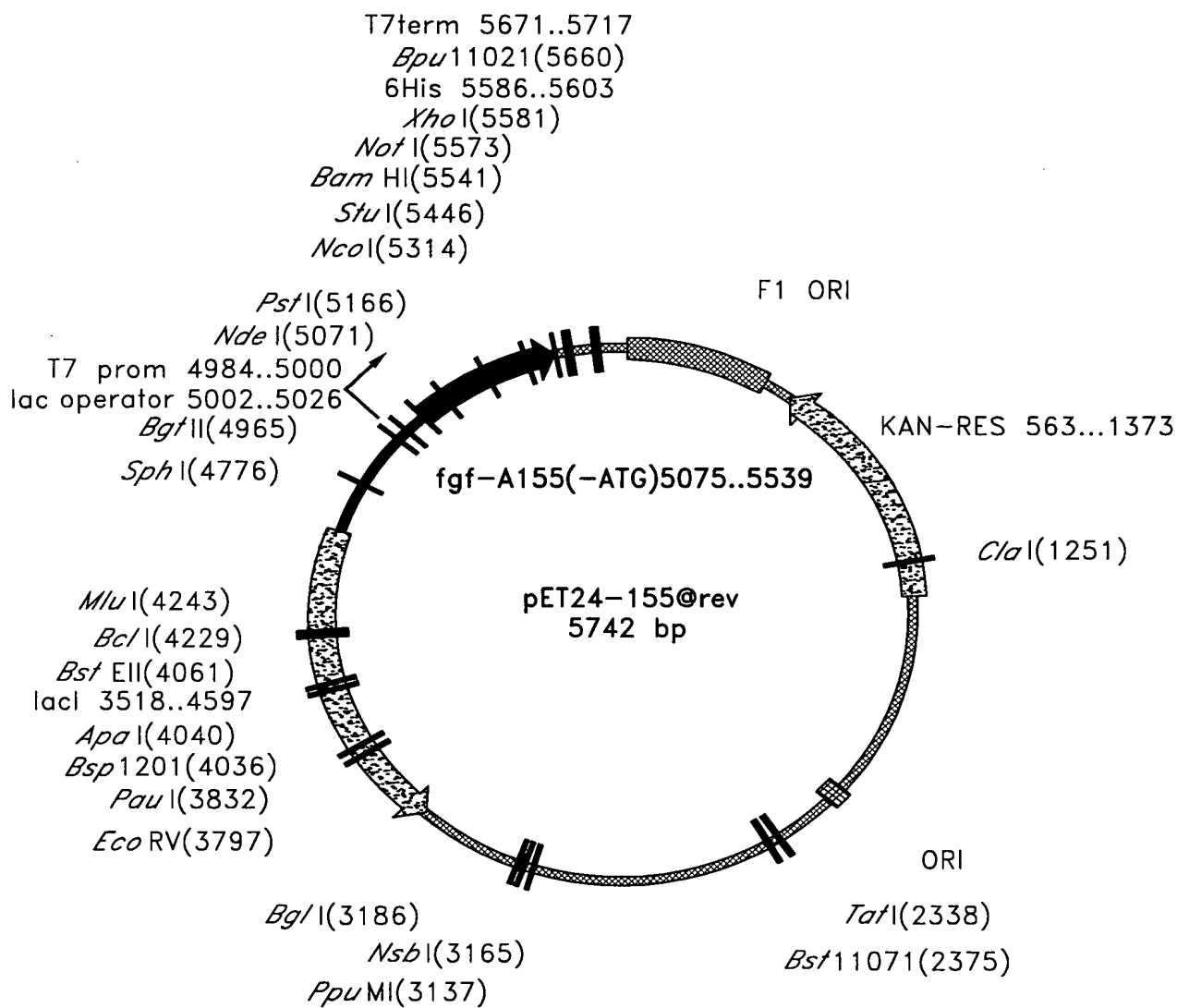


FIG. 3

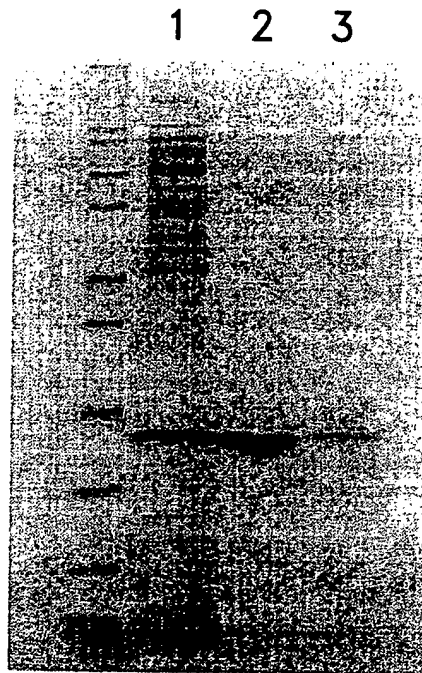
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FGF-1(154aa) PURIFICATION



LANE 1: CRUDE MEDIA: YIELD: 225 mg FGF-1/LITER

2: HEPARIN-SEPHAROSE COLUMN

3: HPLC: C-18 COLUMN

OVERALL PURIFICATION YIELD: 65%

BIOACTIVITY: EQUIPOTENT WITH FGF-1 (SIGMA CHEM) IN:

- 1) 3T3 CELL PROLIFERATION ASSAY, AND
- 2) RAT HIND LIMB ANGIOGENESIS ASSAY

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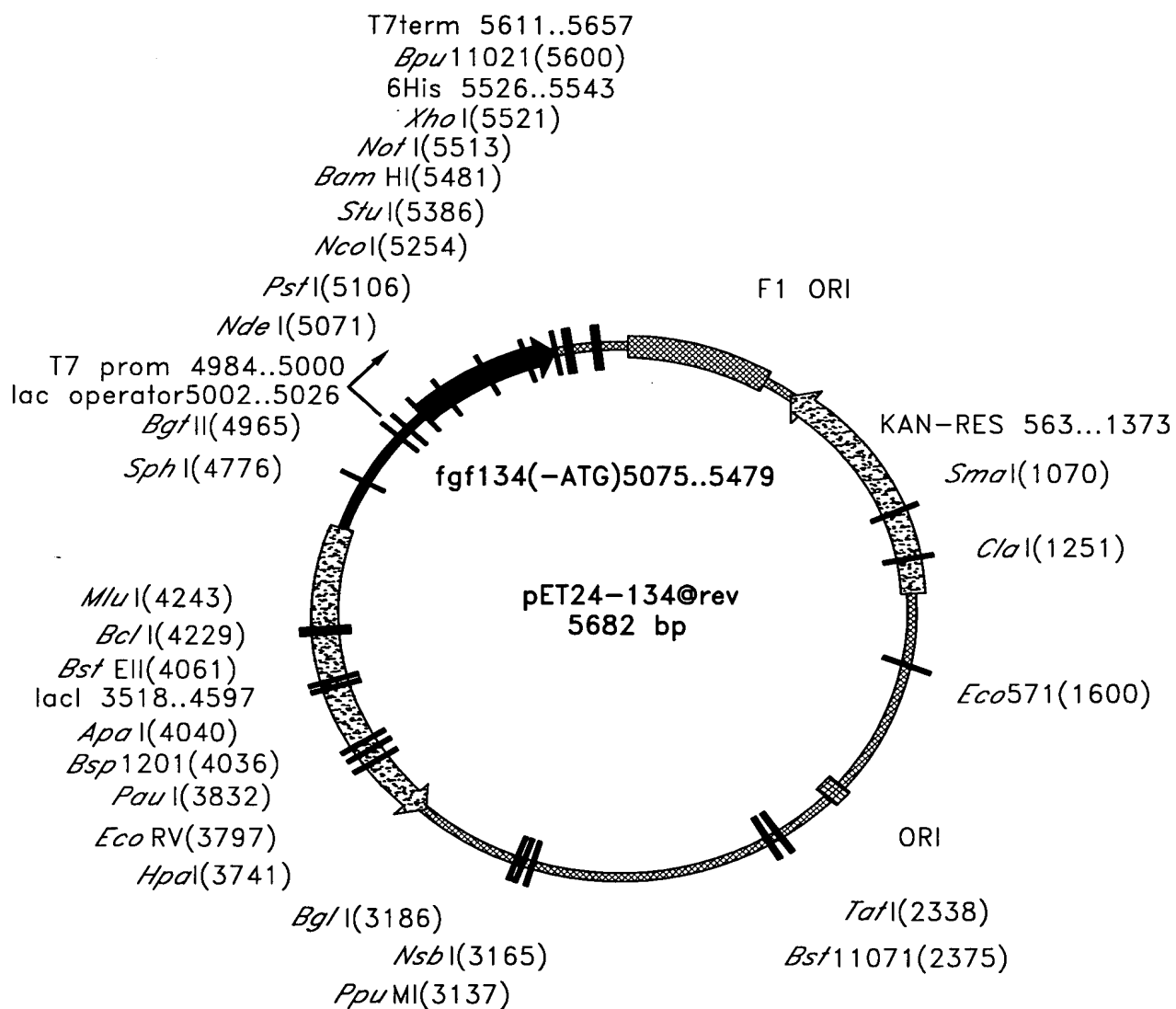


FIG. 5

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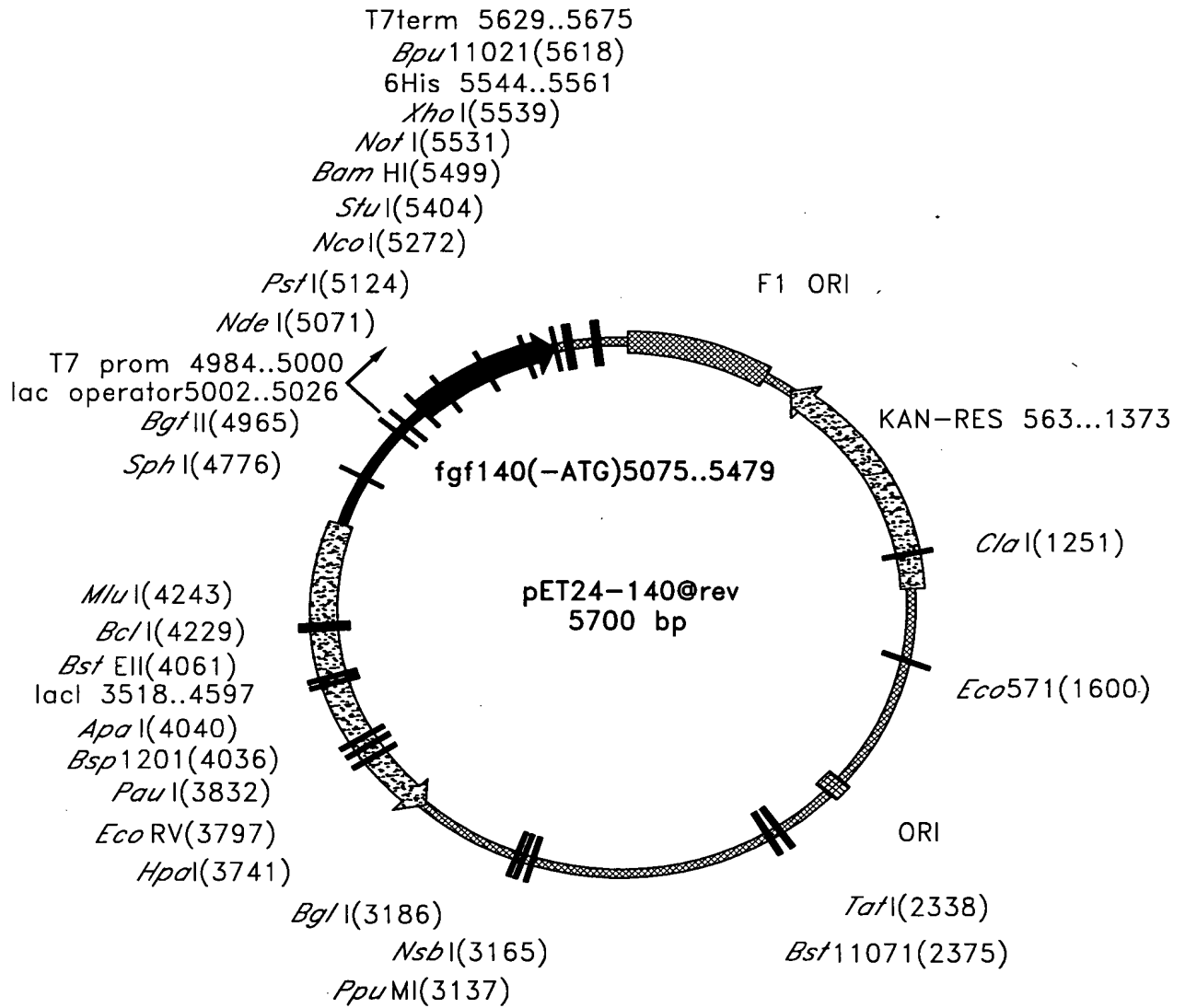


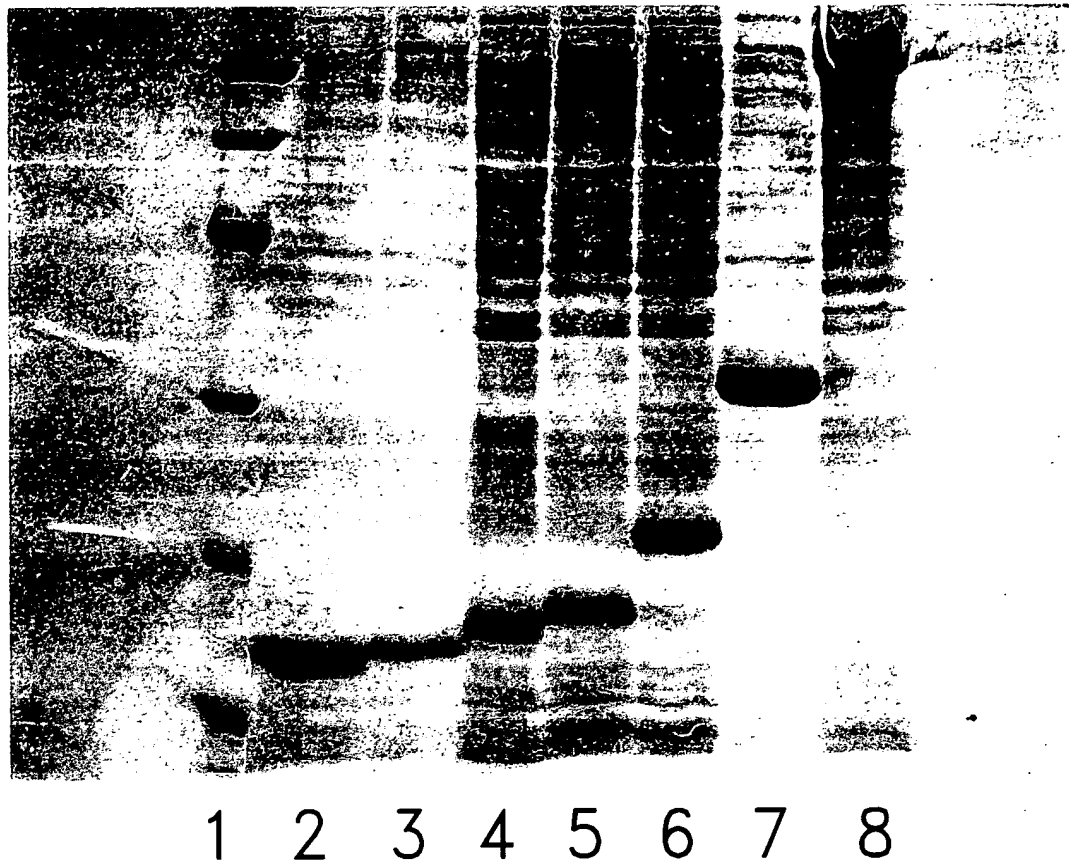
FIG. 7

Appl. No.: Unknown Atty Docket: CVGENG.008CPI

BsaBI		BglII		VspI		BfmI		MbiI		XbaI	
4951	CGCTAGAGGATCGAGATCTCGATCCCGCGAAATTAATACGACTCA	CGCATCTCCTAGCTCTAGAGCTAGGGCGCTTTAATTATGCTGAGT	CTATAGGGGAATTGTGAGCGGATAACAATTCCCCTCTAGAAATAA	GATATCCCCTTAACACTCGCCTATTGTTAAGGGGAGATCTTTATT							
+2	MetPheAsnLeuPro		ProGlyAsnTyrLysLysProLysLeuLeuTyrCysSerAsnGln								
5041	TTTTGTTTAACTTTAAGAAGGAGATATACATATGTTTAACTTCC	AAAACAAATTGAAATTCTTCTCTATATGTATACAAATTGGAAGG	GCCCCGGAATTACAAAAACCAAGCTTCTTTACTGCAGTAACGG	CGGGCCCTTAATGTTTTTTGGGTTCTGAAGAAATGACGTCATTGCC							
+2	yGlyHisPheLeuArgIleLeuProAspGlyThrValAspGlyThr		ArgAspArgSerAspGlnHisIleGlnLeuGlnLeuSerAlaGln								
5131	AGGACACTTCTCTGCGAATTCTGCCAGATGGCACAGTAGATGGGAC	TCCTGTGAAGGACGCTTAAGACGGTCTACCGTGTCATCTACCCGTG	TCGCGATCGCTCCGACCAGCACATTACAGTGCAACTCTCGGCCGA	AGCGCTAGCGAGGCTGCTCGTGAAGTCGACGTTGAGAGCCGGCT							
+2	uSerValGlyGluValTyrIleLysSerThrGluThrGlyGlnTyr		LeuAlaMetAspThrAspGlyLeuLeuTyrGlySerGlnThrPro								
5221	AAGCGTTGGAGAGGTCTATATCAAGTCGACGGAGACTGGCCAGTA	TTCGCAACCTCTCCAGATATAGTTACAGTGCCTCTGACCGGTCAT	CCTTGCCATGGACACCGATGGGCTTCTGTATGGCTCAGACAGCC	GGAACGGTACCTGTGGCTACCCGAAGACATACCGAGTGTCTGCGG							
+2	oAsnGluGluCysLeuPheLeuGluArgLeuGluGluAsnHisTyr		AsnThrTyrIleSerLysLysHisAlaGluLysAsnTrpPheVal								
5311	TAACGAAGAATGCTTGTGTTTCTAGAAAAGACTAGAAGAAAACCATTA	ATTGCTTCTTACGAACAAAGATCTTTCTGATCTTCTTTTGGTAAT	CAACACGTACATATCGAAAAACATGCAGAGAAGAACTGGTTTGT	GTTGTGCATGTATAGCTTTTTTGTACGCTCTCTCTTGACCAAACA							
+2	iGlyLeuLysLysAsnGlySerCysLysArgGlyProArgThrHis		TyrGlyGlnLysAlaIleLeuPheLeuProLeuProValSerSer								
5401	AGGCCTTAAAAAAATGGTTCTGTAAAGCGTGGACCACGGACTCA	TCCGGAATTTTTTTTACCAAGGACATTTCGCACCTGGTGCCTGAGT	CTATGGCCAAAAGGCTATCTTGTTCCTGCCACTACCAGTGAGCTC	GATACCGGTTTTTCCGATAGAACAAGGACGGTGATGGTCACTCGAG							
+2	rAsp***										
5491	CGACTAAGGATCCGAATTCGAGCTCCGTCGACAAGCTTGC GGCCG	GCTGATTCCTAGGCTTAAGCTCGAGGCAGCTGTTTCAACGCCGGC	CACTCGAGCACCACCACCACCACCACCCTGAGATCCGGCTGCTAACA	GTGAGCTCGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGT							

FIG. 8

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ELECTROPHOREGRAMM

1-MOLECULAR WEIGHT KIT (94 000,67 000,43 000,30 000
20 100, 14 4000)

THE CULTURAL MEDIUM, CONTAINING:

2-haFGF 134(40 μ I OF THE CULTURAL MEDIUM)

3-haFGF 140(40 μ I OF THE CULTURAL MEDIUM)

4-IFN α 2B(40 μ I OF THE CULTURAL MEDIUM)

5-haFGF 155(40 μ I OF THE CULTURAL MEDIUM)

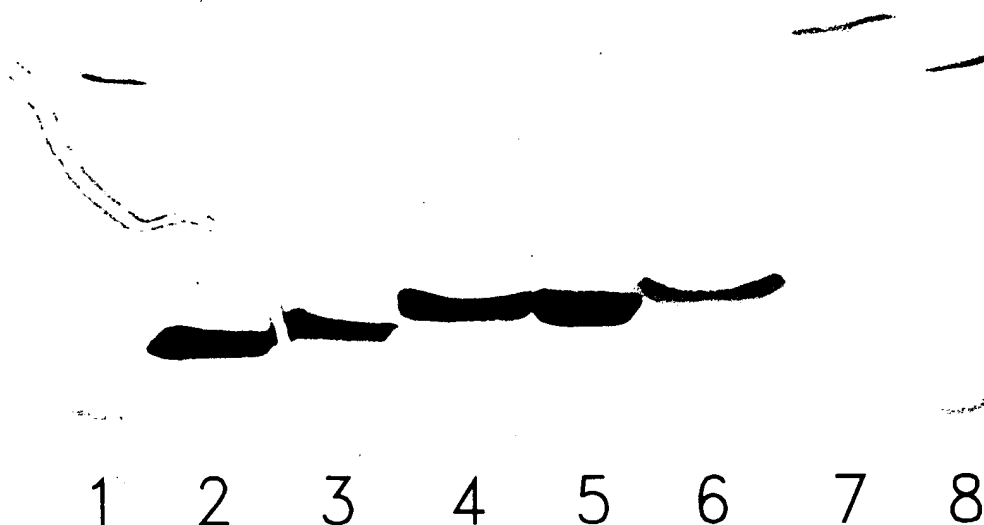
6-HGH(40 μ I OF THE CULTURAL MEDIUM)

7-MAP(40 μ I OF THE CULTURAL MEDIUM)

8- β -GALACTOSIDASE OF E. COLI(40 μ I OF THE CULTURAL MEDIUM)

FIG. 9

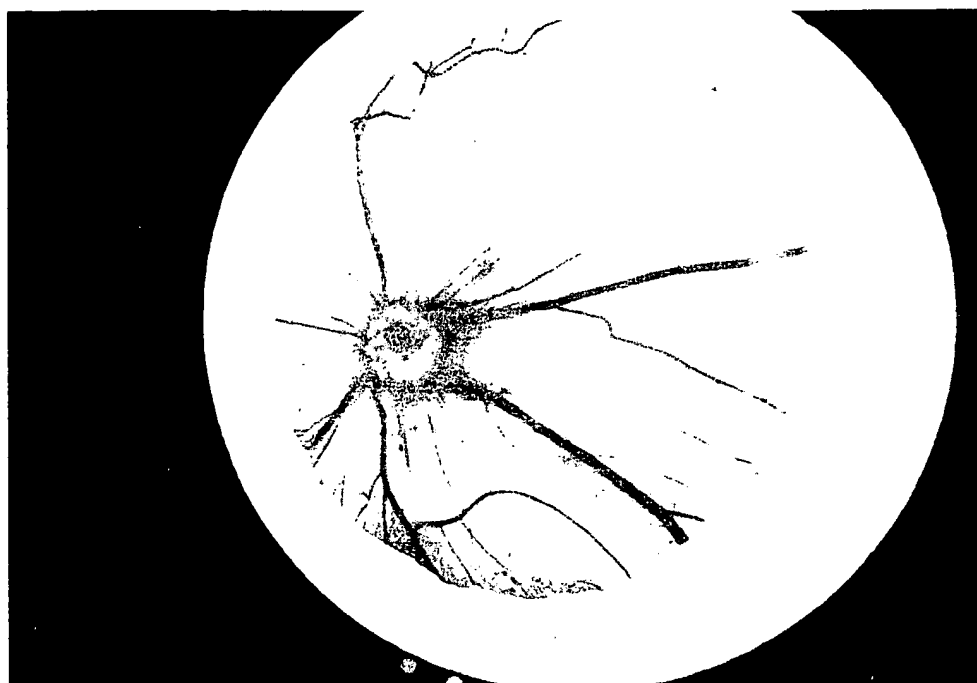
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ELECTROPHOREGRAMM OF THE PURIFIED PRODUCTS:

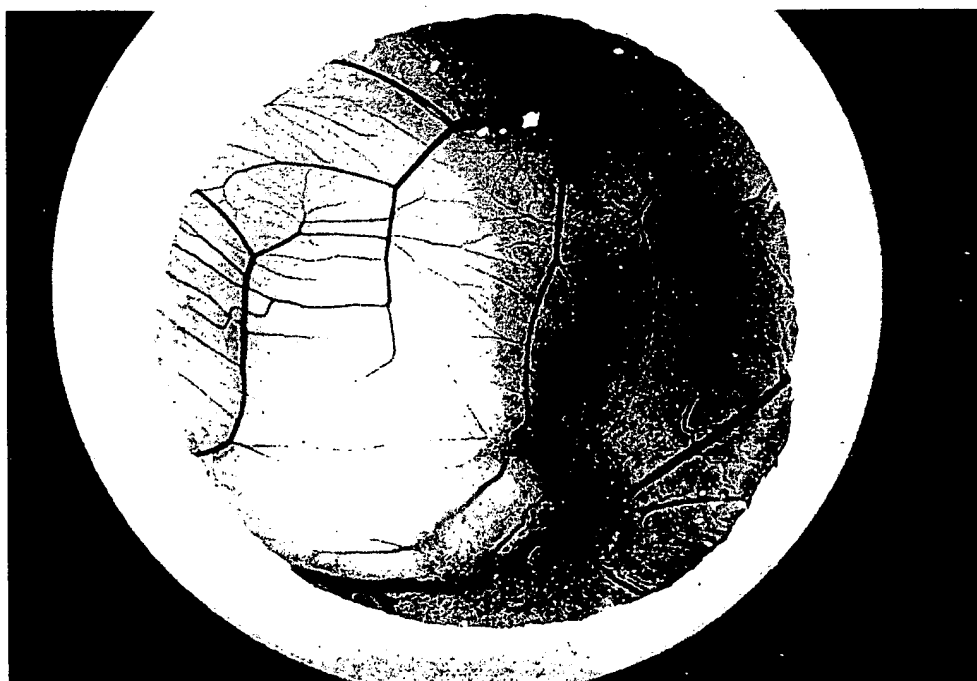
- 1-MOLECULAR WEIGHT KIT (94 000,67 000,43 000,30 000
20 100, 14 4000)
- 2-haFGF 134
- 3-haFGF 140
- 4-haFGF 146
- 5-IFN α 2B
- 6-haFGF 155
- 7-MAP
- 8-MOLECULAR WEIGHT KIT

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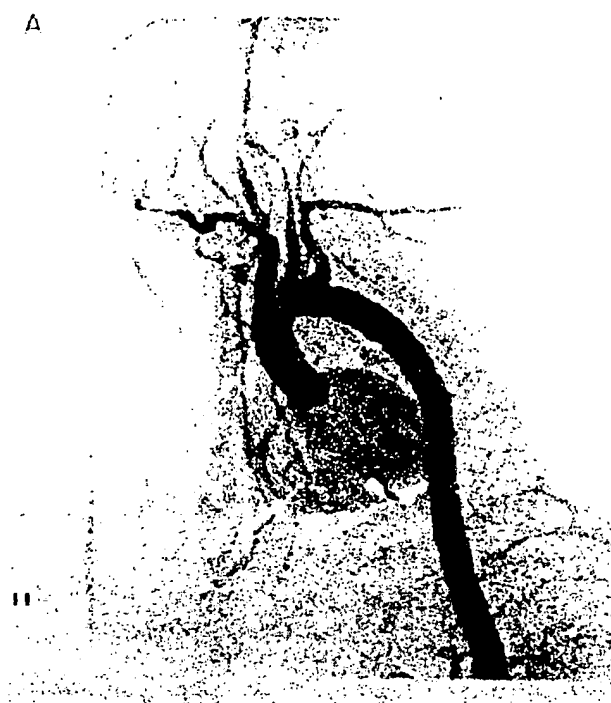
1 ug/ml recombinant haFGF 155

FIG. 11A



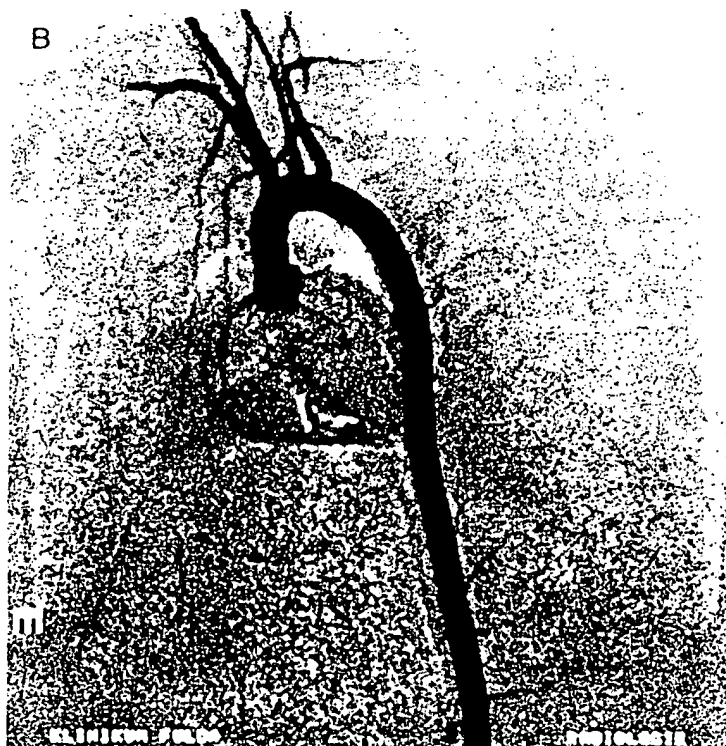
CONTROL

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10 pgHBGF 1

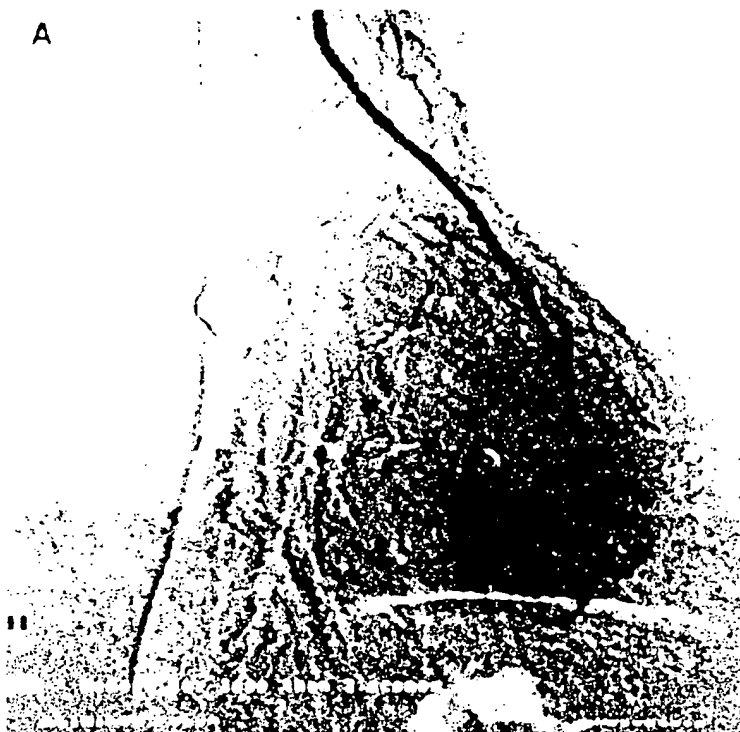
FIG. 12A



without HBGF 1

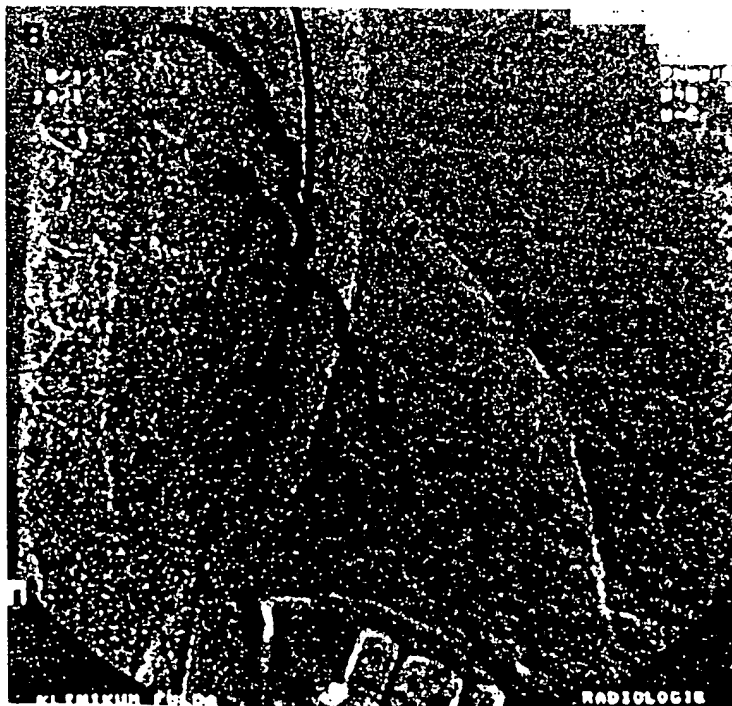
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A



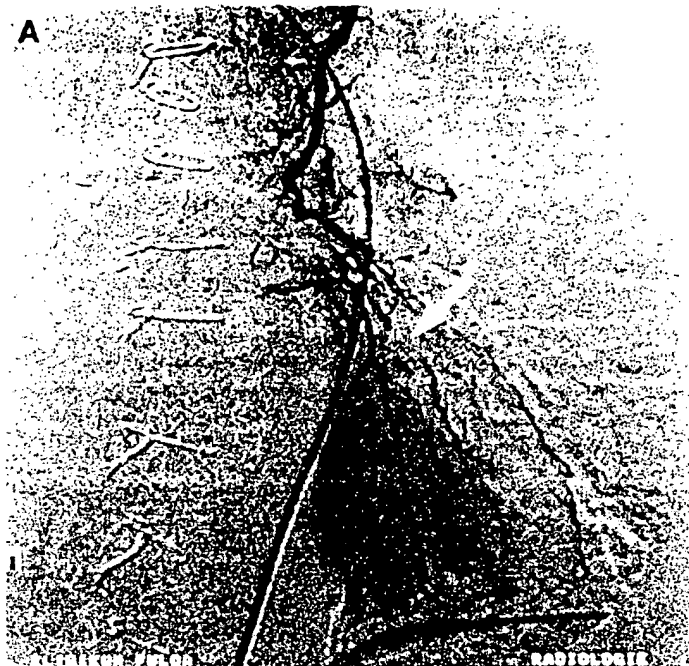
10 ug/kg HBGF-1

FIG. 13A



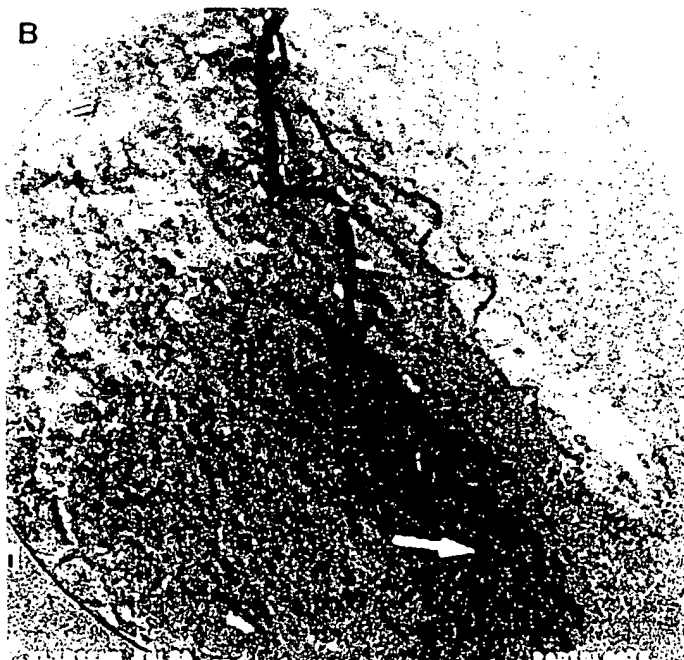
without HBGF-1

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10 ug/kg HBGF-1

FIG. 14A



10 ug/kg HBGF-1

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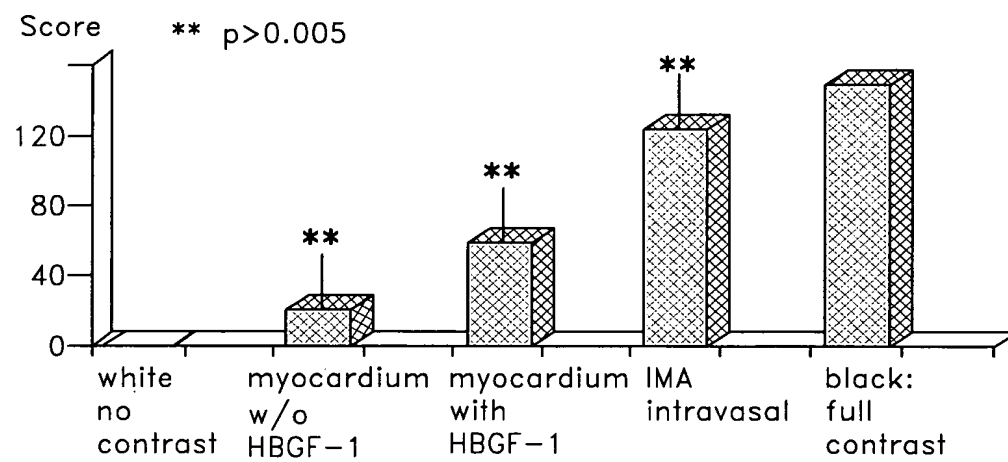


FIG. 15

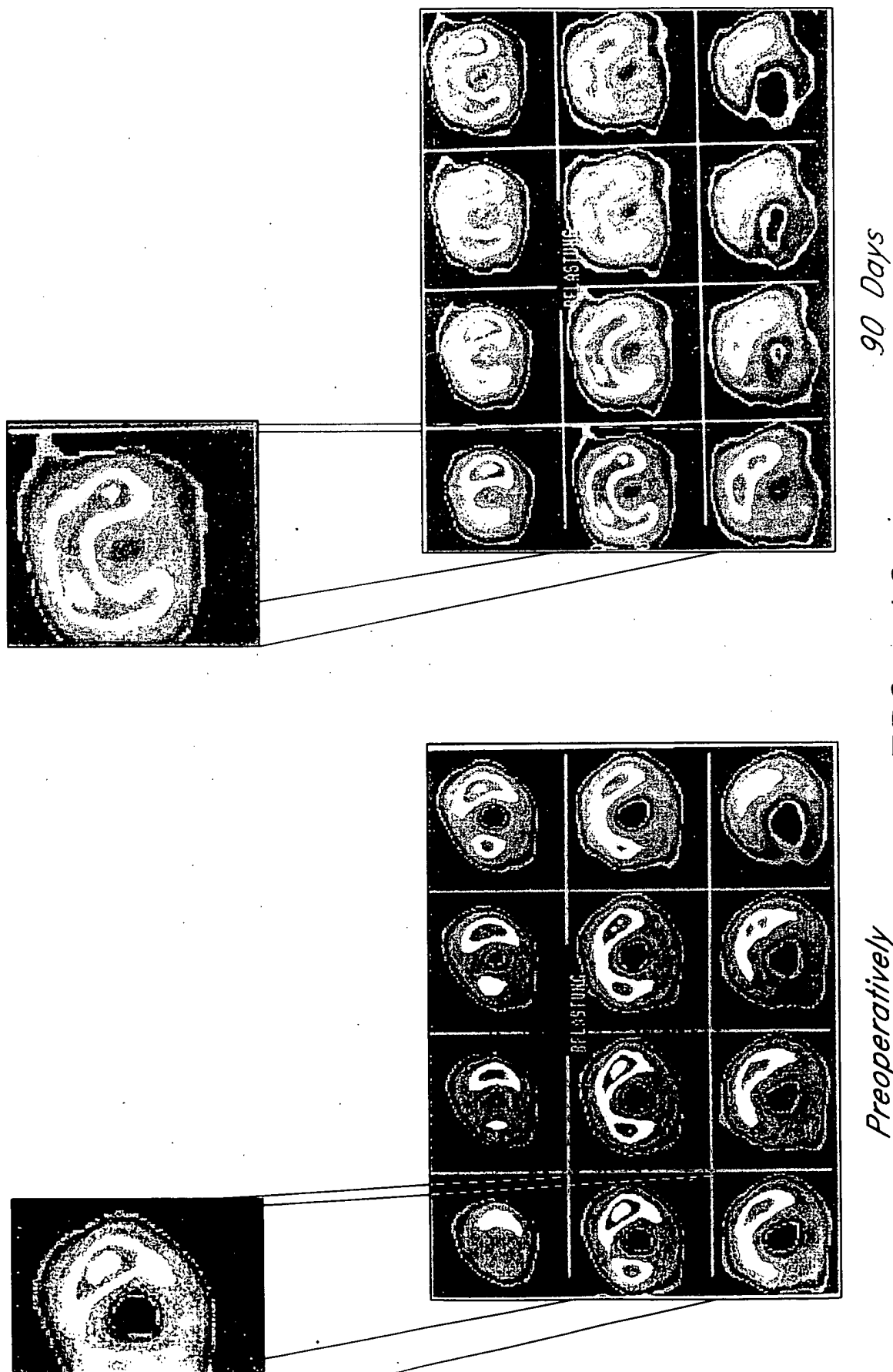


FIG. 16

FIG. 17A

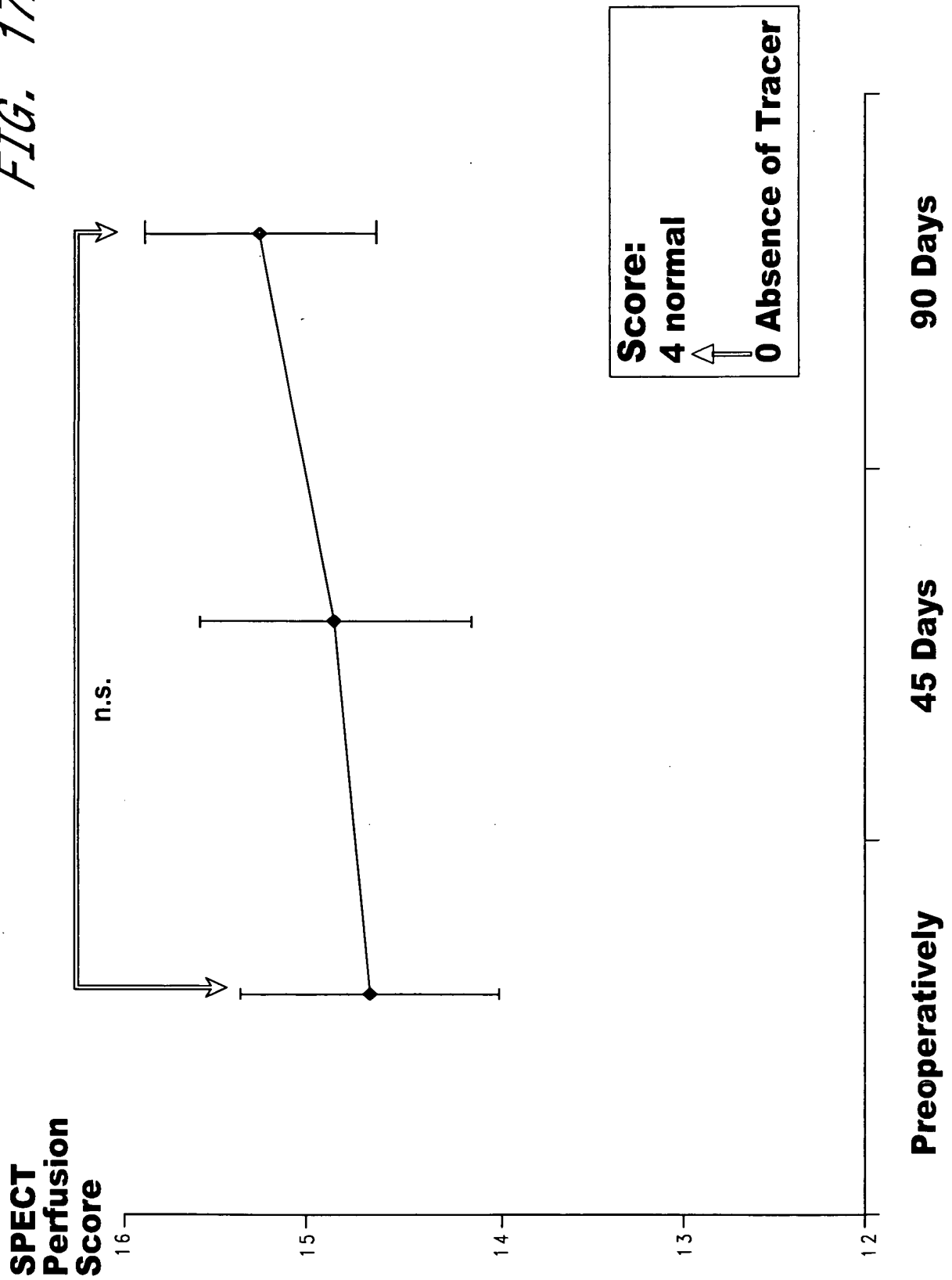


FIG. 17B

